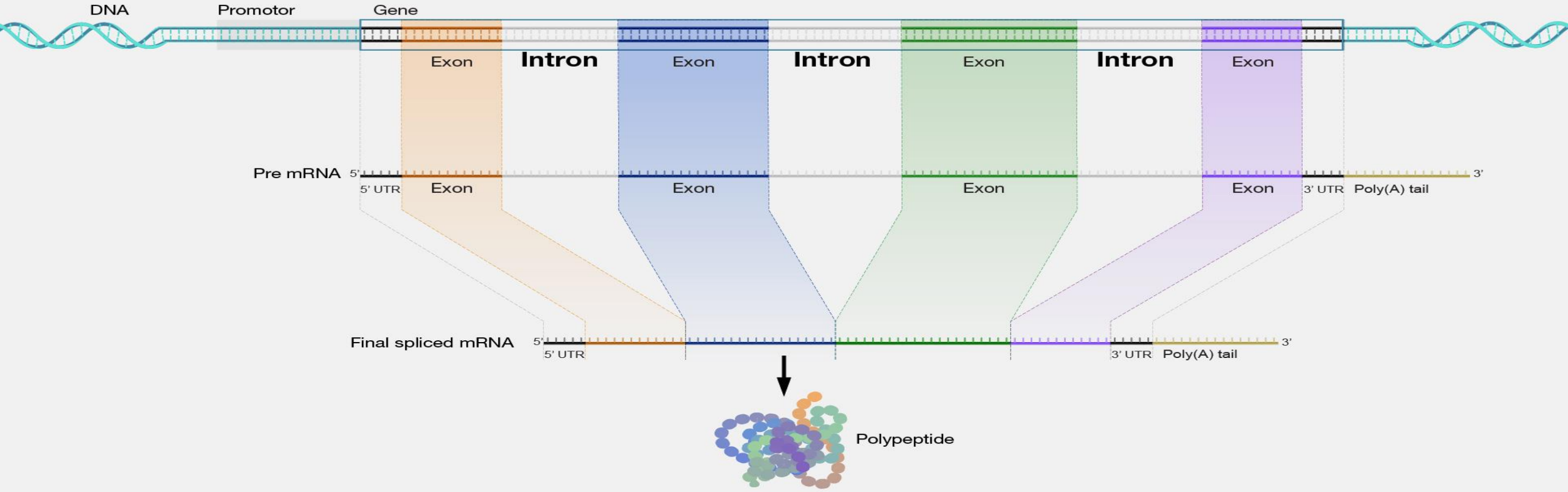
Post-transcriptional regulation(Modification)



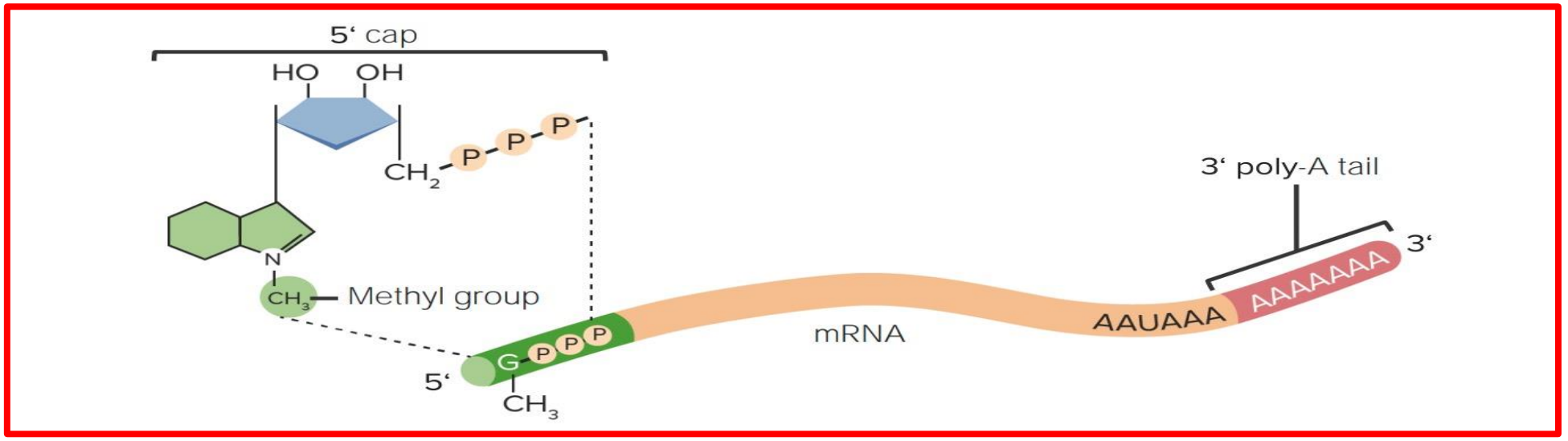
Post-transcriptional regulation(Modification)

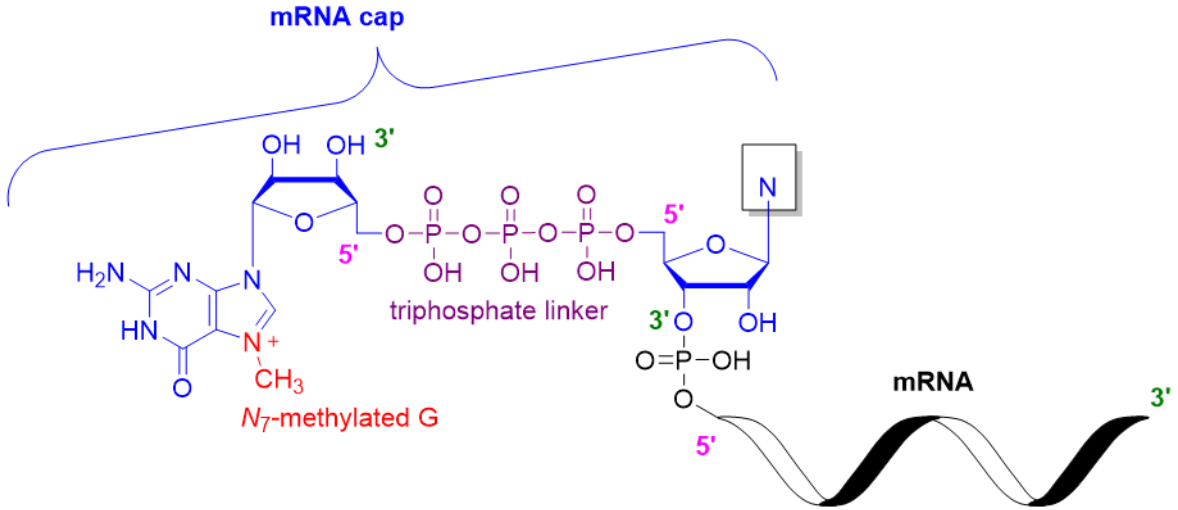
- Post-transcriptional regulation can be used to regulate the active amount of RNA by modification. It occurs between the transcription phase and the translation phase of gene expression, each of transcript RNA type (mRNA, rRna and tRNA) will subjected to modification events after ending transcription.
- In prokaryotic cell, RNA transcripts are ready to act as mRNAs and get translated into proteins right away, but in Eukaryotic cell, pre-mRNa needs to go through a few more steps to become an actual mRNA.

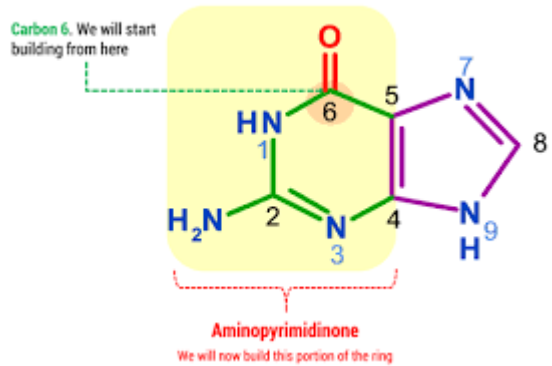
These modifications include:-

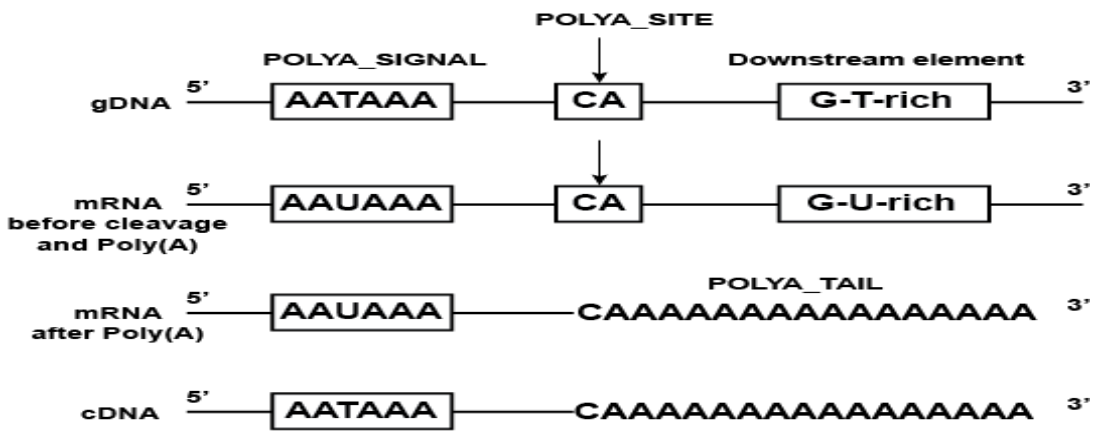
- 1- processing includes Additions of 5' cap and poly-A tail. Both the cap and the tail protect the transcript and help it get exported from the nucleus and translated on the ribosomes (protein-making "machines") found in the cytosol
- A- Adding cap structure: A 7-methylguanosine cap is added to the 5’ end of the growing transcript by a phosphate linkage.
- The cap protects the transcript from being broken down and helps the ribosome attach to the mRNA and start reading it to make a protein.
- B- Polyadenylation (poly Adenine residue): Once elongation is complete, the pre-mRNA is cleaved by an endonuclease between an **AAUAAA consensus sequence** and a **GU-rich sequence**, leaving the AAUAAA sequence on the pre-mRNA.

- An enzyme called poly(A) polymerase then adds a string of approximately 100 - 200 A residues, called the poly-A tail. The tail makes the transcript more stable and helps it get exported from the nucleus to the cytosol.




mRNA cap
OH
OH
3'
5'
5'
triphosphate linker
N
3'
OH
5'
mRNA
3'
N_7-methylated G

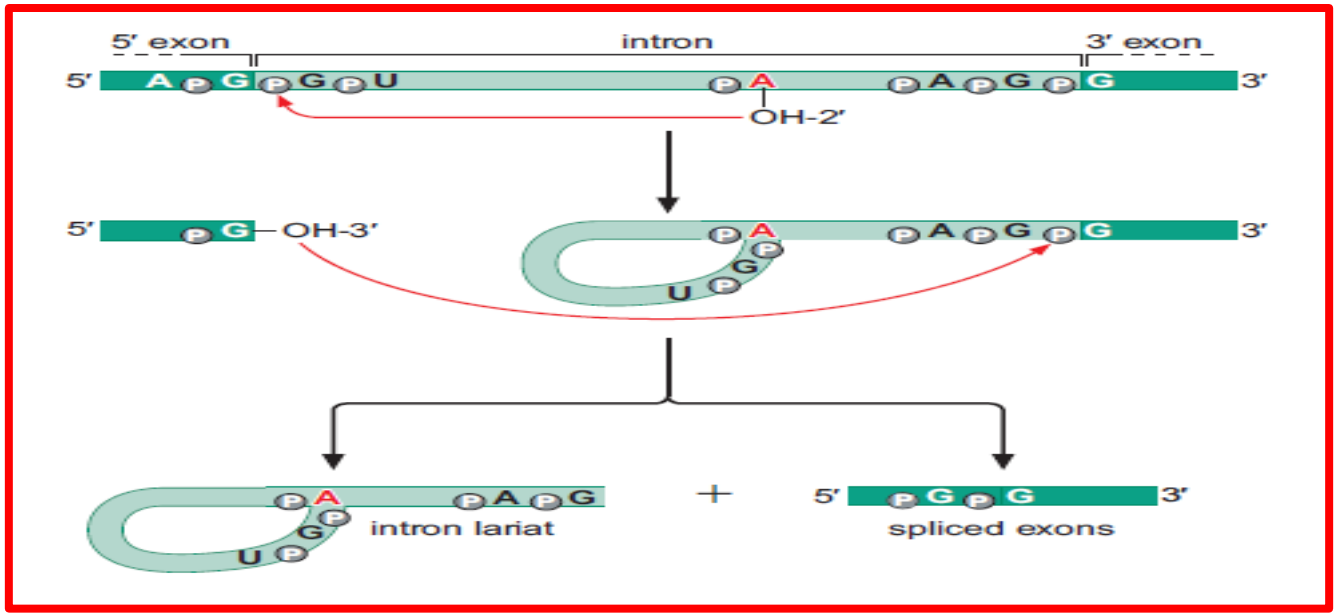

Carbon 6. We will start building from here
Aminopyrimidinone
We will now build this portion of the ring


POLYA_SITE
POLYA_SIGNAL
Downstream element
gDNA
5'
AATAAA
CA
G-T-rich
3'
mRNA
before cleavage
and Poly(A)
AAUAAA
G-U-rich
POLYA_TAIL
mRNA
after Poly(A)
CAAAAAAAAAAAAAAAA
cDNA

- **2- splicing :** Eukaryotic genes are composed of exons, which correspond to protein-coding sequences (**exon** signifies that they are expressed), and intervening sequences called (sequences in mRNA do not encode
- functional proteins). **introns**
- **The process of removing introns and reconnecting exons is called splicing.**
- Introns are removed and degraded while the pre-mRNA is still in the nucleus.
- The splicing process is catalyzed by protein complexes called spliceosomes
- that are composed of proteins and RNA molecules called small nuclear RNAs
- (snRNAs).
- Spliceosomes recognize sequences at the 5′ and 3′ end of the intron.
- Usually the transcript mRNA is shorter than the origin gene it-self.

Splicing includes:-

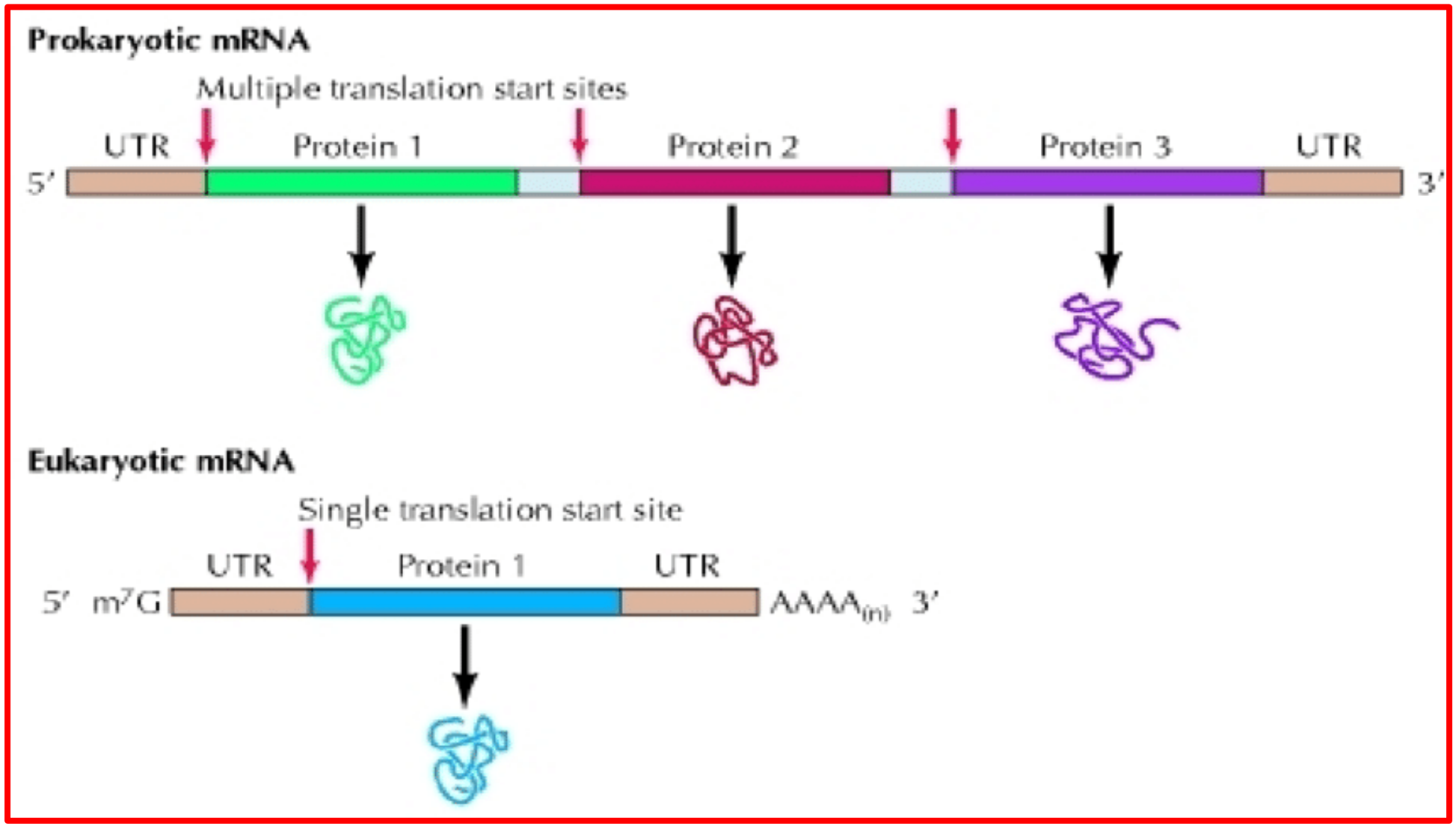
- 1-The first cleavage occur by splicesome machine at 5́ end of the intron region rich with GU residue
- 2- Then the intron bend back to form lariat structure via 5́→3́ phosphodiester bond.
- 3- Cleavage at 3́ end of the intron to completely released
- 4- Joining the exons by ligase enzyme to give arise to mature mRNA .



- Mature mRNA is a single-stranded RNA molecule, carries information from DNA to the ribosome, the sites of protein synthesis (translation) in the cell.
- The coding sequence of the mRNA determines the amino acid sequence in the protein .

The basic differences in mRNA structure in prokaryotic and eukaryotic

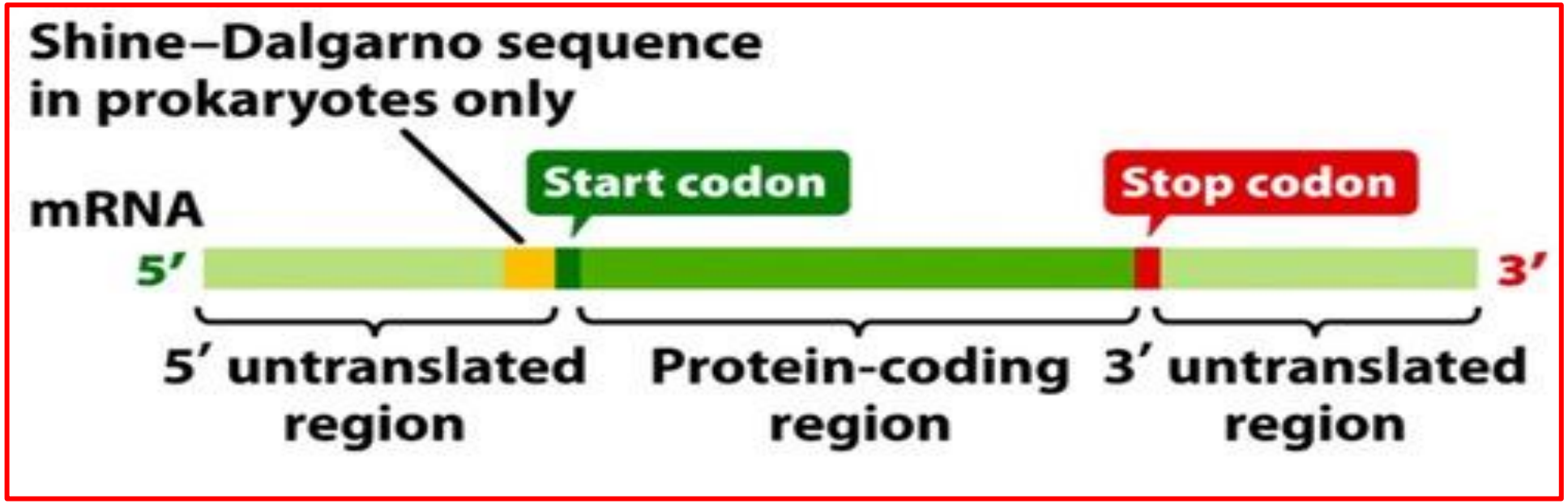
- The **main difference** between prokaryotic and eukaryotic mRNA is that prokaryotic mRNA is **Polycistronic** ,whereas eukaryotic mRNA is **Monocistronic**. Furthermore, several structural genes of an operon are transcribed into a single mRNA while eukaryotic mRNA contains a single gene transcribed into an mRNA molecule.

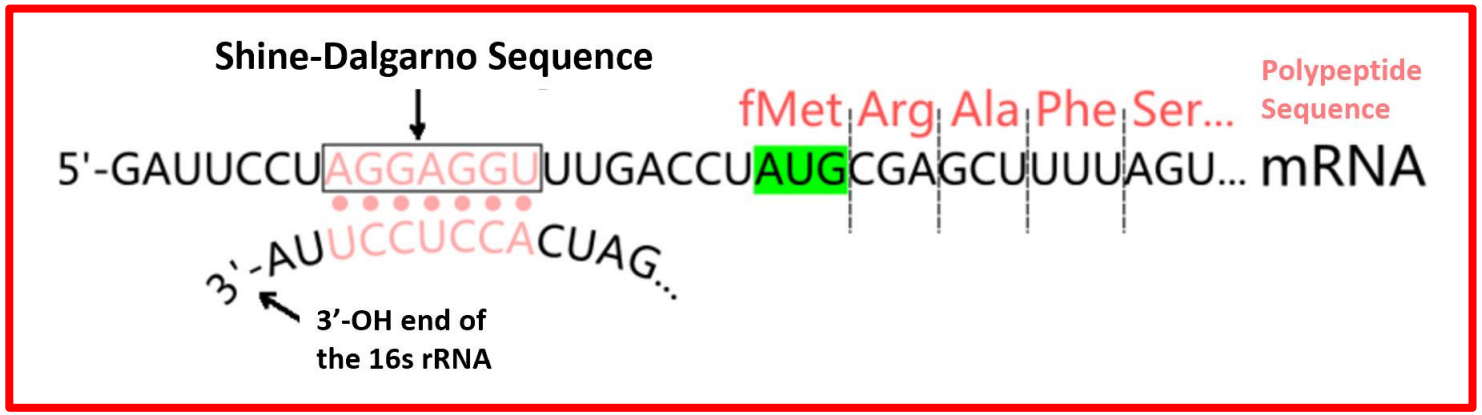

Prokaryotic mRNA
Multiple translation start sites
UTR
Protein 1
Protein 2
Protein 3
UTR
5′
3′
Eukaryotic mRNA
Single translation start site
UTR
Protein 1
UTR
5′
m7G
AAAA(n)
3′

- (2) prokaryote mRNA is very short lived in *E. coli* half-lives range from 10s-20min. Therefore it is degraded primarily after translation has been initiated.
- Short life span of mRNA enables prokaryotes to synthesize different proteins or enzymes in response to changes in the external environment. On other hand, Eukaryote mRNAs are more stable in yeast half-lives range from 1 – 60 min.

- Prokaryotic transcription occurs in the cytoplasm alongside translation. Prokaryotic transcription and translation can occur simultaneously. This is impossible in eukaryotes, where transcription occurs in a membrane-bound nucleus while translation occurs outside the nucleus in the cytoplasm

- 4) In prokaryotes the primary mRNA transcript is functional as soon as it is synthesized. In eukaryotes, however, the RNA transcript must undergo processing before it is a functional mRNA. This processing occurs in the nucleus and involves three steps: 5′ capping, 3′ polyadenylation (polyA tailing), and exon splicing.
- (5) in prokaryotic mRNA cells start (5̀ end) with Leader sequence (upstream
- region; 5′ UTR) that contains a ribosome binding site (RBS), also known as
- the Shine–Dalgarno sequence, which is usually 3–10 base pairs upstream
- from the initiation codon.
- The mRNA ends (3′ end) with un-translated region (3′-UTR or tailer sequence) is the section of mRNA that immediately follows the translation termination codon. The 3′-UTR often contains regulatory regions that post-transcriptionally influence gene expression.


Shine–Dalgarno sequence
in prokaryotes only
mRNA
Start codon
Stop codon
5′
3′
5′ untranslated region
Protein-coding region
3′ untranslated region

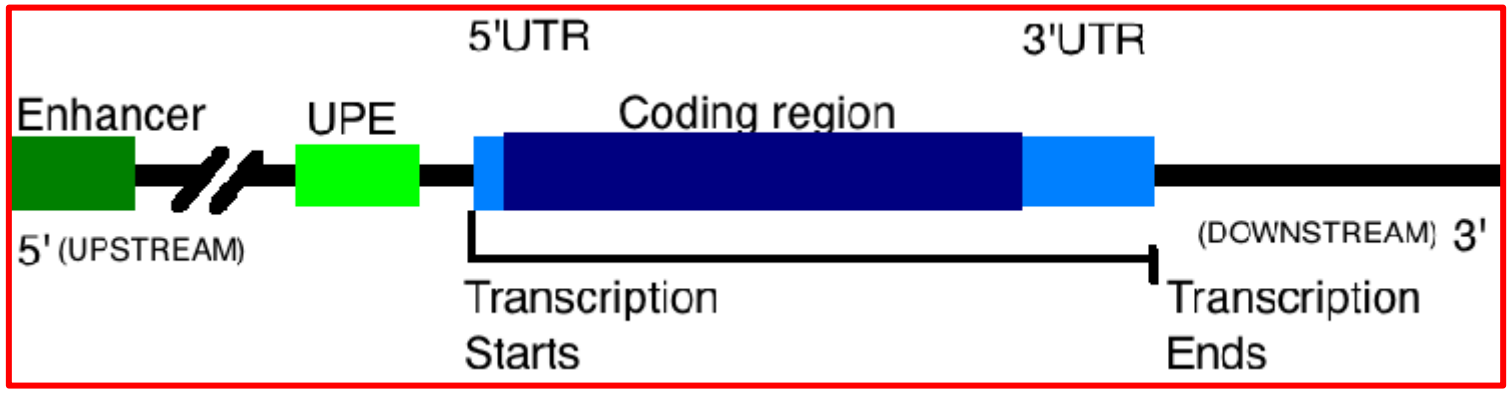

Shine-Dalgarno Sequence
fMet Arg Ala Phe Ser...
Polypeptide Sequence
5'-GAUUCCUAGGAGGUUUGACCUAUGCGAGCUUUUAGU...
mRNA
3'-AUUCCUCCACUAG...
3'-OH end of the 16s rRNA

Shine–Dalgarno (SD) sequence

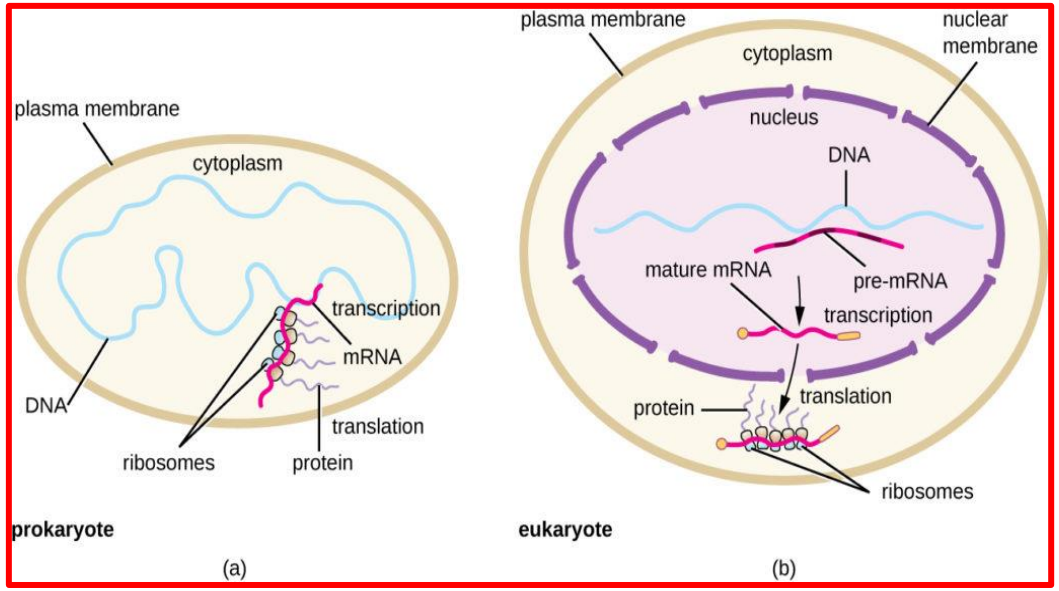
- Is a ribosomal binding site, generally located around 8 bases upstream of the start codon AUG. The RNA sequence helps recruit the ribosome to the messenger RNA (mRNA) to initiate protein Synthesis by aligning the ribosome with the start codon.
- The Shine–Dalgarno sequence is common in bacteria, but rarer in archaea .It is also present in some chloroplast and mitochondria transcripts. The six-base consensus sequence is **AGGAGG**; in *Esherichia coli* for example, the sequence is AGGAGGU.
- The Shine–Dalgarno sequence was proposed by Australian scientists John Shine and Lynn Dalgarno in 1973.

- Most eukaryotic 5'UTR region includes the Kozak consensus sequence (ACCAUGG) which contains the start codon (AUG) to initiate translation.

- The eukaryotic 5’ UTR also contains cis-acting regulatory elements called upstream open reading frames (uORFs) and upstream AUGs (uAUGs) and termination codons, which have a great impact on the regulation of translation



Modification of Ribosomal RNA (rRNA)


plasma membrane
cytoplasm
DNA
transcription
mRNA
translation
ribosomes
protein
prokaryote
(a)
plasma membrane
nuclear membrane
cytoplasm
nucleus
DNA
mature mRNA
pre-mRNA
transcription
protein
translation
ribosomes
eukaryote
(b)